REMARKS

Claims 76-82 are currently pending in this application. Claims 78, 81, and 82 are withdrawn from consideration. Claims 76, 77, 79, and 80 are rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement. Claims 76, 77, and 80 are rejected under 35 U.S.C. § 102(b) for anticipation by Jacob et al. (Proc. Nat'l. Acad. Sci. USA 87:968-972, 1990; hereinafter "Jacob") in view of Anderson et al. (Ann. Rev. Immunol. 23:447-485, 2005; hereinafter "Anderson"). Finally, claims 76, 77, 79, and 80 are rejected for obviousness-type double patenting over claim 9 of U.S. Patent No. 6,660,487 and provisionally rejected for obviousness-type double patenting over claims 16-30, 65-73, and 91-108 of copending U.S. Serial. No. 10/851,983. By this reply, Applicants cancel claims 79-82, amend claims 76, 77, and 78, and address each of the rejections.

Support for the Amendment

Support for the amendment to claim 56 may be found in the specification at least at, e.g., page 6, line 21, through page 7, line 3, page 18, lines 3-8, page 19, lines 7-13, and page 29, line 19, through page 30, line 8, as well as prior claims 79 and 80. Support for the amendment to claim 77 may be found in the specification at least at, e.g., page 19, lines 7-13. Claim 78 is amended for reasons related to clarity. No new matter is added by the amendment.

Rejoinder of Claim 78

Applicants respectfully request rejoinder of present claim 78 under 37 C.F.R. § 1.104. Present claim 78 depends from and includes all of the limitations of present independent claim 76, as is required to achieve such rejoinder. Applicants respectfully request acknowledgement from the Office that present claim 78 has been rejoined.

Interview with Examiners Belyavskyi and Skelding

Inventor Faustman and Applicant's representatives wish to thank Examiners Belyavskyi and Skelding for the courtesy of an in-person interview on November 19, 2007. The enablement and anticipation rejections of claims 76, 77, 79, and 80 were discussed. During the interview,

Examiner Belyavskyi acknowledged that additional data from the inventor, Dr. Faustman, would likely overcome the enablement rejection with respect to the treatment of those autoimmune diseases supported by the data. As was discussed during the interview, Dr. Faustman's additional data, discussed below, show that TNF-α and a TNF-α receptor agonist antibody, agents which bind to the cell-surface TNF-α receptor and activate the NFκB signaling pathway, support the treatment of diabetes, rheumatoid arthritis, multiple sclerosis, scleroderma, Crohn's disease, Grave's disease, psoriasis, Celiac disease, adult-onset idiopathic hypoparathyroidism (AOIH), Sjögren's syndrome, and Addison's disease by killing autoreactive immune cells in these autoimmune disease patients.

Applicants believe that present claims 76, 77, and 78 are in condition for allowance and respectfully request that the Office contact the undersigned by telephone in order to resolve any remaining issues in this case should the Office disagree.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 76, 77, 79, and 80 are rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement. The Office states:

> the specification, while being enabling for a method of treating pre-clinical type I diabetes in non-obese diabetic mice does not reasonably provide enablement for treating overt type I diabetes in [sic: or] any autoimmune disease in any mammal. (Office Action, p. 2.)

Applicants have cancelled claims 79 and 80 and have amended present claims 76, 77, and 78 to recite a method of treating a human exhibiting symptoms of an autoimmune disease selected from the group consisting of diabetes, rheumatoid arthritis, multiple sclerosis, scleroderma, Crohn's disease, Grave's disease, psoriasis, Celiac disease, adult-onset idiopathic hypoparathyroidism (AOIH), Sjögren's syndrome, and Addison's disease by administering an agent that binds to a cell-surface receptor, thereby activating the NFκB signaling pathway. The data provided in the Declaration of Dr. Faustman, which was filed on June 15, 2007, and discussed in detail by the Office in the present Office Action, and the inventor's data discussed below, which was presented during the in-person interview, fully support the enablement of present claims 76, 77, and 78.

I. Applicants' *in vitro* and *in vivo* Data Support the Enablement of Present Claims 76, 77, and 78

Applicants have demonstrated that NFκB precursor proteins are not processed properly in the NOD mouse resulting ineffective signaling within the NFκB pathway after TNF-α exposure and failure of anti-apoptotic gene expression. Applicants have discovered that the ineffective signaling within the NFκB pathway is at least partially responsible for the susceptibility of autoreactive T-cells to killing by TNF-α and TNF-α receptor agonists. Using *in vitro* and *in vivo* assays, Applicants have demonstrated that TNF-α and TNF-α receptor agonists specifically eliminate autoreactive T cells responsible for autoimmune disease. Through a series of experiments, discussed in detail below and in the attached publications, Applicants have confirmed that the methods of present claims 76, 77, and 78 are enabled, as described in the present specification, to treat diabetes, rheumatoid arthritis, multiple sclerosis, scleroderma, Crohn's disease, Grave's disease, psoriasis, Celiac disease, adult-onset idiopathic hypoparathyroidism (AOIH), Sjögren's syndrome, and Addison's disease.

NF-kB Precursor Proteins Are Not Processed Properly in the NOD Mouse

Applicants discovered that signaling proteins in the NF-kB pathway of autoimmune NOD mice are defective. Applicants' data show that in the NOD mouse with TNF-α stimulation there are normal, basal levels of proteins in the NFκB pathway, yet these NFκB pathway proteins do not become active (i.e., they are not proteolytically cleaved) following stimulation of the TNF-α signaling pathway. Thus, normal signaling of the NFκB pathway does not occur in NOD mice (see Hayashi and Faustman, Mol. Cell. Biol. 19:8646-8659, 1999; a copy is attached).

Proteosomes from NOD Mice Are Missing a Key Subunit

In the NOD mouse, Applicants discovered that TNF-α stimulation of T cells fails to properly activate signaling proteins within the NFkB pathway. This pathway is dependent on an intact proteasome (i.e., a multi-catalytic protein complex that cleaves various NFkB proteins), which is defective in CD8+ T cells from NOD mice. Applicants' data support the conclusion

that one reason TNF-α kills a subset of NOD CD8+ T cells is that these highly activated T cells require proper activation of the NFkB pathway to survive exposure to TNF-α. These cells lack the ability to cleave IkB, which is required for activation of NFkB. This defect in turn creates defective proteasome complex subunits, rendering these autoreactive cells vulnerable to killing by exposure to TNF-α or TNF-α receptor agonists (see Hayashi and Faustman, *supra*).

TNF-α Kills the Disease-Causing Cells

Applicants showed that TNF-α induces a dose-dependent decrease in the survival of autoreactive T cells from male or female NOD mice. In particular, Applicant demonstrated that a subset of T cells from the spleen (i.e., the autoreactive T cells) die with low dose TNF-α treatment (see, e.g., Figure 5; Hayashi and Faustman, *supra*).

Applicants also conducted a cell transfer experiment to show that stimulation of the TNF-α pathway in NOD mice eliminates autoreactive CD8+ T cells. The experimental materials and methods are disclosed in Kodama et al. (Cell. Mol. Life Sci. 62:1850-1862, 2005; hereinafter "Kodama I"; a copy of which is provided herewith). The experiment involved transferring autoreactive T cells from NOD mice with disease, with or without pre-treatment of the autoreactive T cells with TNF-α, to young NOD mice without disease.

Splenocytes from TNF-α and Class I Treated NOD Mice Can No Longer Transfer Disease

Applicants showed that autoreactive T cells rapidly transfer disease to young NOD mice in the absence of pre-treatment with TNF-α (Figure 4, left panel; Kodama I, *supra*), but that brief treatment of these autoreactive T cells with TNF-α removes sufficient numbers of autoreactive T cells to greatly hamper autoimmune disease transfer (Figure 4, right panel; Kodama I, *supra*).

In Vivo Induction of TNF-α Induces Islet Clearance and T-cell Death

Applicants also carried out TUNEL staining of NOD pancreas at various times after NOD mice had had one treatment with a TNF-α inducer. By day 2, the invasive insulitis remained on the islet, but the staining showed that autoreactive T cells had been killed (Figure 2; Kodama I).

TNF-α Reverses Established Diabetes in NOD Mice

Applicants have also demonstrated that TNF-α reverses established autoimmune disease in NOD mice. These results are described in Ryu et al. (J. Clin. Invest. 108:63-72, 2001) and Kodama et al. (Science 302:1223-1227, 2003; hereinafter "Kodama II"), copies of which are provided.

Agonism of the TNF-α Pathway Kills Auto-Reactive CD8+ T cells (CTLs) but not CD4+ T cells

Applicants' specification teaches the use of agents that activate the NFκB signaling pathway to bring about killing of autoreactive immune cells involved in autoimmune disease. Applicants have shown that activation of the NFκB signaling pathway using, e.g., TNF-α and a TNF-α receptor agonist, targets a specific subset of autoreactive CD8+ T cells that are responsible for mediating human autoimmune disease. In Kühtreiber et al. (J. Immunol. Methods 306:137-150, 2005; a copy is provided herewith), Applicants describe isolating splenocytes from diabetes-prone NOD and control mice and identifying a quantifiable subpopulation of T cells, with co-expression of CD8, that selectively undergo cell death on exposure to TNF-α (see Results, §§ 3.3-3.5, pages 142-146; Kühtreiber et al., *supra*). As is clearly disclosed in Kühtreiber et al., only the subset of CD8+ T cells undergo cell death in response to TNF-α agonism (see, e.g., page 146, col. 1).

Moreover, Applicants determined that this subpopulation of autoreactive splenic CD8+ T cells is only present in NOD mice that develop disease; autoreactive splenic CD8+ T cells are absent in NOD mice that do not develop disease. Applicants exposed splenic CD8+ T cells from NOD mice that develop diabetes and invasive insulinitis to a TNF-α agonist and observed the death of these cells, whereas the exposure of splenic CD8+ T cells from NOD mice that never become diabetic and only develop peripheral, not invasive, insulinitis, which is a hallmark of nonprogression of autoimmune disease, to a TNF-α agonist does not result in cell death (see page 146, § 3.6; Kühtreiber et al., *supra*). Applicants' data reveal the presence of a specific subpopulation of autoreactive T cells (i.e., CD8+ T cells) that cause autoimmune disease and that are sensitive to TNF-α agonists, which promote the death of these cells.

TNF-α pre-treatment of T cells from diseased NOD mice prevents disease transfer to healthy naïve NOD mice

The adoptive transfer techniques discussed above have also been used by Applicants to confirm not only the identify of a specific subpopulation of CD8+ T cells that are responsible for causing autoimmune disease in NOD mice, but also that these CD8+ T cells can be selectively killed. Applicants have demonstrated that even a single treatment of splenocytes that include CD8+ T cells from a diseased NOD mouse with a TNF-α agonist delays for several months the appearance of autoimmune disease in healthy NOD mice that receive transfers of the treated splenocytes (see page 15 of the previous Reply, as well as ¶ 8 and Exhibit C of the accompanying Declaration).

In the present Office Action, the Office states "[w]ith respect to section 8, there is either insufficient experimental details and/or insufficient data provided to understand the significance of the statements made in this section or the new data of Exhibit C" (Office Action, p. 6). In response, Applicants direct the Office to Kodama I, *supra*, which describes in detail the adoptive transfer experiments mentioned in the previous Reply and Declaration (see, e.g., pages 69-70). Applicants also provide a brief description of the technique and Applicants' results. Briefly, adoptive transfer involves obtaining donor splenocytes from newly diabetic female NOD mice not treated with CFA or TNF-α, treating the donor splenocytes *in vitro* with TNF-α for 24 hours prior to administration, and administering (i.e., adoptively transferring) the treated donor splenocytes to normal male NOD mice that have been subjected to irradiation. Following this protocol, Applicants observed the onset of diabetes in *all* of the male NOD mice that received untreated donor splenocytes as early as 10 days post administration. In contrast, 4 out of 5 of the male NOD mice that received treated donor splenocytes remained normoglycemic for at least 40 days and, in some instances, for several months following the cell transfer. Applicants' data reveal that a specific subpopulation of CD8+ T cells are responsible for causing autoimmune disease in NOD mice and that this population of autoreactive T cells can be selectively killed by exposure to TNF-α or a TNF-α agonist.

Treatment of diseased NOD mice with a TNF-α inducer eliminates autoreactive T cells and treats disease

Applicants have also demonstrated that *in vivo* treatment of diseased NOD mice with a TNF-α inducer, CFA, eliminates autoreactive T cells responsible for destructive insulitis of diabetic mice (see, e.g., Fig. 2 of Kodama I, *supra*). As is discussed in Kodama I, Applicants showed that the administration, to female NOD mice, of an agent that induces expression of TNF-α rapidly killed the CD8+ T cells responsible for destructive insulitis and diminished insulitis by day 11. The diminished insulinitis persisted through day 21 and Applicants did not observe any adverse effects to the underlying pancreatic islet structure (see, e.g., Fig. 2A of Kodama I, *supra*). In contrast untreated NOD mice exhibited destruction of islets by extensive and invasive lymphoid infiltrates by day 11 and progressively greater insulitis over the course of 21 days. This data further confirm not only the presence of a specific subpopulation of autoreactive T cells (i.e., CD8+ T cells) that cause autoimmune disease, but also the effectiveness of *in vivo* treatment of autoimmune disease using TNF-α and TNF-α agonists, which promote the death of autoreactive CD8+ T cells.

II. Successful *in vitro* Results Using Human Cells Confirm that the NOD Mouse Model is Predictive of Success in Treating Autoimmune Disease in Humans

Applicants have conducted *in vitro* experiments with cells from human patients diagnosed with one of several different autoimmune diseases and have confirmed that the results in NOD mice discussed above also occur in humans. Namely, TNF-α and TNF-α receptor agonists that stimulate the TNF-α signaling pathway cause the killing of a subpopulation of autoreactive CD8+T cells involved in autoimmune disease in humans. Applicants' evidence that autoreactive CD8+T cells from human autoimmune disease patients are sensitive to cell killing by exposure to TNF-α and TNF-α receptor agonists confirms that the method of present claims 76, 77, and 78 can be used to treat multiple different and diverse autoimmune diseases in humans.

Applicant's data shows that peripheral blood lymphocytes taken from human patients

diagnosed with one of several different autoimmune diseases, in particular, type I diabetes, lupus, psoriasis, Crohn's disease, Graves' disease, Sjögren's syndrome, hypothyroidism, celiac disease, rheumatoid arthritis, and multiple sclerosis, and exposed *in vitro* to TNF-α and TNF-α receptor agonists undergo apoptosis, while cells taken from humans that are not diagnosed with autoimmune disease do not undergo apotosis (see, e.g., Figs. 1-4 and SI Fig. 2 of Ban et al., manuscript submitted for publication (2008); a copy is provided).

Thus, Applicant's data confirm that TNF-α and TNF-α receptor agonist antibodies can be used to treat autoimmune disease in humans by killing autoreactive CD8+T cells that cause the disease.

Summary

In summary, Applicants' data support the full scope of the method of present claims 76, 77, and 78, which can be performed successfully to treat a mammal exhibiting symptoms of autoimmune disease by administering an agent, such as TNF-α or a TNF-α receptor agonist, which binds to a cell-surface receptor and activates the NFκB signaling pathway. Accordingly, the enablement of claims 76, 77, and 78 has been demonstrated. For this reason, Applicants respectfully request that the rejection of claims 76, 77, and 78 under 35 U.S.C. § 112, first paragraph, for lack of enablement be withdrawn.

Rejections under 35 U.S.C. § 102(b)

Claims 76, 77, and 80 are rejected under 35 U.S.C. § 102(b) for anticipation by Jacob in view of Anderson. The Office states that "Jacob teaches that TNF-α is effective at preventing diabetes in non-obese diabetic mice…Jacob is inherently teaching the treatment of prediabetic mice with TNF-α" (Office Action, p. 11). Applicants have amended present independent claim 76 to recite a method of treating a human exhibiting symptoms of autoimmune disease. Jacob, in contrast, only describes administering TNF-alpha to pre-diabetic mice, as acknowledged by the Office. Anderson, which only describes features of disease development and progression in the NOD mouse, fails to remedy the deficiencies of Jacob. This rejection can now be withdrawn.

Obviousness-Type Double-Patenting Rejection

Claims 76, 77, 79, and 80 are rejected for obviousness-type double patenting over claim 9 of U.S. Patent No. 6,660,487, and provisionally rejected for obviousness-type double patenting over claims 16-30, 65-73, and 91-108 of copending U.S. Serial. No. 10/851,983. When the pending claims are found to be otherwise allowable except for these grounds of rejection, Applicants will address the rejections, including consideration of whether to file a terminal disclaimer.

CONCLUSION

In view of the above remarks, Applicants respectfully submit that the claims are in condition for allowance, and such action is respectfully requested.

Enclosed is a Petition to extend the period for replying to the Office Action for two months, to and including July 13, 2008. Please charge Deposit Account No. 03-2095 in the amount of $230.00 for the fee required by 37 C.F.R. § 1.17(a). If there are any other charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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